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Modeling of leaf area of three Afromontane forest tree species through linear measurements

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ABSTRACT:

Leaf area is an important parameter for evaluating growth, development and competitive ability of forest trees. Destructive methods of leaf area determination often require substantial financial investment and technical knowledge, both of which are unavailable in some developing countries and most parts of others. The aim of this study was to develop linear regression models for the non-destructive determination of leaf area of three Afromontane forest tree species (*Cordia millenii* Bak., *Gmelina arborea* Roxb. and *Entandrophragma angolense* (Welw.) C.DC.). Leaf samples were collected from trees in the Tubah Upland Forest in the Bamenda Highlands of Cameroon. Leaf length (L), maximum leaf width (W), and leaf area (LA) were determined. Linear regression analysis was conducted between LA as dependent variable and L, W, LW, L^2, W^2, L^2W, LW^2 as independent variable and F-test was used to test for significance of the model. Goodness-of-fit was evaluated from the coefficient of determination (r^2) and Root Mean Square Error (RMSE). Values of predicted leaf area were then plotted against those of the observed leaf area. The findings showed that the leaf area of *C. millenii*, *G. arborea* and *E. angolense* can be reliably determined from the models LA = 471.59 + $0.10LW^2$ (r^2 = 0.85, RMSE =170.91, p = 0.00), LA = -972.63 + 110.90W (r^2 = 0.92, RMSE = 102.72, p = 0.00), and LA = -31.81 + 2.88LW (r^2 = 0.97, RMSE = 66.00, p = 0.00), respectively. Further studies involving additional formulae derived from L and W are, however, encouraged.

Keywords:

Leaf size, Leaf dimensions, Montane forest, Linear regression.

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INTRODUCTION

The Cameroon highlands ecoregion comprises of highland forests and grassland patches mainly above 900 m elevation scattered along the border area between Cameroon and Nigeria (WWF, 2017). Along an altitudinal gradient, the vegetation changes from submontane to montane forests and ultimately subalpine grassland. Apart from having one of the highest levels of endermism among vascular plants in Africa (Ndenecho, 2011), the forests provide a wide range of ecosystem services. For instance, they constitute a vital source of medicine, food, wildlife forage, building material, and raw materials for rural artisan industries. In addition, the highlands form essential water catchments. However, most of the forest that once totally covered the Bamenda Highlands component of the ecoregion has been cleared. In fact, more than 50% of the forest cover has been lost since the 1960s (Ernest Vunan, pers. comm.). The forests are found on fertile volcanic soils, which in combination with adequate rainfall, have attracted a lot of farmers. The high human population density in the region has subjected the remaining forest to enormous pressure of cultivation and grazing. The degradation of the forests by humans is likely to be accentuated by changing global climatic conditions. The protection of the forest remnants, development of adaptive strategies to climate change, and restoration of degraded portions of the ecosystem are imperative for a sustainable supply of the benefits that are contained in the Bamenda Highlands biome.

Leaf area is an important determinant of plant performance in open and closed forests. The parameter is indicative of light interception, growth (Ghoreishi *et al.*, 2012) and plant competitive ability in the field (Zadeh *et al.*, 2011). It is an essential component of plant growth analysis and often used in plant ecophysiology research (Lambers *et al.*, 2008). Leaf area is routinely measured in experiments where some physiological phenomena like photosynthesis, respiration, water and nutrient uptake, and transpiration are of interest (Calvo-Alvardo *et al.*, 2008). Several techniques have been presented for leaf area determination which can, however, be classified as either destructive or non-destructive (Marshall, 1968). Destructive methods are those that require the harvesting of leaves at the time of evaluation. They are performed with the use of automated leaf area meters, planimeters, scanners, and image processing software (Easlon and Bloom, 2014) among others. However, some of the equipment require substantial financial investment and a high level of technical competence for operation and maintenance (Ugese *et al.*, 2008), both of which may not be readily available in some developing countries and most parts of others. Non-destructive techniques do not require defoliation and are based on regression models that estimate leaf area from linear measurements of leaf length (L), maximum leaf width (W), L^2, W^2 or various combinations of these variables (Chanda and Singh, 2002; Çirak *et al.*, 2005; Ilkaee *et al.*, 2011; Ogoke *et al.*, 2015). The leaf area estimation models can provide researchers with many advantages. First, they constitute an alternative to the high cost and complexity associated with destructive techniques while also maintaining a high level of accuracy (Ghoreishi *et al.*, 2012). Second, leaf area measurements can be carried out on the same plants for the entire duration of a study (José *et al.*, 2014), minimizing experimental variability (De Swart *et al.*, 2004). Though fast and reliable, dealing with a large number of leaves can result in destructive sampling techniques being more time-consuming and laborious than using mathematical models for leaf area determination (Mokhtarpour *et al.*, 2010; Ilkaee *et al.*, 2011).

Cordia millenii Bak. (Boraginaceae), *Gmelina arborea* Roxb. (Verbanaceae), and *Entandrophragma angolense* (Welw.) C.DC. (Meliaceae) are tree species of great ecological and economic importance (Adam and Krampah, 2005; Tchinda, 2008; Jiofack, 2010) in the Bamenda highlands that have experienced notable declines in population. For instance, only *ca* 200 acres are left of the hitherto *ca* 400 acres of Tubah Upland forest (Ndimuh, 2017) that constituted a major habitat for the trees within the Bamenda highlands. There is a general lack of automated and digital equipment for measuring leaf area in the region. Given that the trait is quite valuable in evaluating plant acclimation to field conditions (Lambers *et al*., 2008), its determination from regression equations is crucial for the putting in place of suitable management practices that ensure competitive advantage on regeneration sites and optimize growth of trees in plantations and natural forests. To the best of our knowledge, there are no published works on leaf area prediction models for the species. Consequently, this study was aimed at developing regression equations for the non-destructive estimation of leaf area of *C. millenii*, *G. arborea*, and *E. angolense* from leaf linear measurements.

MATERIALS AND METHODS

Study setting

The site for sample collection was the Tubah Upland Forest of the Bamenda highlands. Tubah Subdivision (4°50' – 5°20'N, 10°35' – 11°59'E; 950 – 1500 m a.s.l.) is located some 15 km from Bamenda, the capital city of the North West Region of Cameroon. The Sub-division covers four main villages; Bambili, Bambui, Kedjom-Keku, and Kedjom-Ketinguh (Nguh and Maluh, 2017). The vegetation is mainly Savannah grassland. Its forested area is found at the northern part, mainly Kedjom-Keku where the samples in this study were obtained. A high population density and unsustainable human behavior related to farming and grazing have largely destroyed the forest and grassland vegetation (Melle *et al*., 2016). Tubah is characterized by a dry (November to April) and a rainy season (May to October). Average annual rainfall varies from 1780 to 2290 mm and most of the precipitation occurs between July and September (Ndenecho, 2010). Average annual temperature is 20.67°C (Yuninui, 1990), with a 20-22°C / 13-14°C maximum / minimum (Kiteh, 2011). Relative humidity is highest during the months of July and August (> 80%) and lowest in January and February (45 to 52%) (Ndenecho, 2010; Ndenecho, 2011).

Study design and data collection

The donors consisted of five trees each of *C. millenii*, *G. arborea*, and *E. angolense*. To capture as much morphological variability as possible for sampling, the vertical axis of the canopy was partitioned into four fractions of approximately similar height. A fully expanded leaf was collected from each of the fractions, resulting in twenty leaves per tree species. Only leaves that had their edges preserved and had not suffered herbivore or insect damage were harvested. After the collection, the leaf was immediately sealed in an airtight polythene bag. All the samples from each tree spe-

Table 1. ANOVA table for regression model

S. No	Source	df	SS	MS	F	*p*-value
1	Regression	1	SSR	$MSR = \frac{SSR}{1}$	$\frac{MSR}{MSE}$	$\Pr\left(F > \frac{MSR}{MSE}\right)$; $F \sim F_{1,n-2}$
2	Error	n-2	SSE	$MSE = \frac{SSE}{n-2}$		
3	Total	n-1	SST			

Table 2. Results of Pearson correlation between observed leaf area and variables from leaf linear measurements

S. No	Species	Variable	L	W	LW	L^2	W^2	L^2W	LW^2
1	*C. millenii*	LA	0.79	0.83	0.92	0.77	0.81	0.86	0.92
2	*G. arborea*	LA	0.94	0.96	0.95	0.93	0.96	0.93	0.95
3	*E. angolense*	LA	0.96	0.95	0.99	0.96	0.96	0.97	0.98

cies were subsequently bulked in a large leak-proof polythene bag and transported to the Pan African Institute for Development – West Africa Support Centre in Bamenda for measurement of leaf area and linear dimensions.

Observed leaf area was obtained with the use of a graph paper. The graph paper consisted of 1 cm^2 cells each subdivided into twenty-five 0.04 cm^2 cells. The leaf was fully spread out on the graph sheet and its margin traced. The observed leaf area was determined from the number of 1 cm^2 and 0.04 cm^2 cells that fell within the boundary of traced region. Leaf length (L: distance from lamina tip to lamina-petiole junction along the midrib) and maximum leaf width (W: widest distance across lamina perpendicular to length) were measured.

Data analysis

The data for each tree species were subjected to Pearson-Moment correlation for determination of the relationship between observed leaf area and leaf linear dimensions (L, W), their squares and products (LW, L^2, W^2, L^2W, LW^2). The data were then subjected to linear regression analysis using the observed leaf area as dependent variable and L, W, LW, L^2, W^2, L^2W, and LW^2 as independent variables. Statistical significance of the regression models was determined from *F*-test (see Table 1). Goodness-of-fit was evaluated from the coefficient of determination (r^2) and Root Mean Square Error (RMSE). Selection of the suitable model for leaf area estimation was based on highest r^2 and least RMSE.

$$\mathrm{SSR} = \sum_{i=1}^{n}(\hat{y}_i - \bar{y})^2$$

$$\mathrm{SSE} = \sum_{i=1}^{n}(y_i - \hat{y}_i)^2$$

$$\mathrm{SST} = \sum_{i=1}^{n}(y_i - \bar{y})^2$$

Table 3. Regression models of relationships between observed leaf area and variables from leaf linear measurements

S. No	Species	Variable	Model	r^2	*p*	RMSE
1	*C. millenii*	L	LA = -255.54 + 67.01L	0.62	0.00	268.82
		W	LA = -1059. 31 + 128.78W	0.67	0.00	243.76
		LW	LA = 98.83 + 2.72LW	0.84	0.00	176.94
		L^2	LA = 594.37 + 1.27L^2	0.60	0.00	277.79
		W^2	LA = 231.63 + 3.14W^2	0.66	0.00	254.38
		L^2W	LA = 650.69 + 0.06L^2W	0.74	0.00	221.41
		LW^2	LA = 471.59 + 0.10LW^2	0.85	0.00	170.91
2	*G. arborea*	L	LA = -401.09 + 66.82L	0.89	0.00	119.54
		W	LA = -972.63 + 110.90W	0.92	0.00	102.72
		LW	LA = 162.52 + 2.17LW	0.91	0.00	109.14
		L^2	LA = 304.65 + 1.50L^2	0.87	0.00	130.21
		W^2	LA = -3.94 + 3.09W^2	0.92	0.00	102.79
		L^2W	LA = 480.96 + 0.06L^2W	0.87	0.00	131.95
		LW^2	LA = 480.42 + 0.08LW^2	0.90	0.00	118.34
3	*E. angolense*	L	LA = -610.43 + 59.29L	0.93	0.00	111.68
		W	LA = -1230.47 + 175.18W	0.90	0.00	127.42
		LW	LA = -31.81 + 2.88LW	0.97	0.00	66.00
		L^2	LA = 168.84 + 1.07L^2	0.91	0.00	121.44
		W^2	LA = -183.15 + 7.10W^2	0.92	0.00	116.46
		L^2W	LA = 341.54 + (-0.06)L^2W	0.94	0.00	102.99
		LW^2	LA = 242.83 + 0.16LW^2	0.97	0.00	75.36

where, ŷ is predicted leaf area; y and ȳ are observed leaf area and the mean, respectively.

A regression plot was established between values of predicted leaf area and those of observed leaf area for the appropriate model for each of the tree species.

RESULTS AND DISCUSSION

The value of Pearson-Moment correlation coefficient (*r*) was positive for the relationship between observed leaf area and each leaf linear trait (L, W, their squares and products) for the three tree species (Table 2). Relationships of the sort have been established previously for neotropical rainforest trees (Brito-Rocha *et al*., 2016), vegetable (Schwarz and Karlring, 2001) and fruit (Ramkhelawan and Brathwaite, 1990) crops. However, the leaf trait(s) that showed the strongest correlation to the observed leaf area was inconsistent across species, suggesting that the leaf area of the tree species cannot be estimated with the use of a common parameter or regression model. There was, however, a general variability in *r* among the tree species (Table 2), reflecting phylogenetic differences in leaf shapes.

Non-destructive estimations of leaf area from leaf dimensions are well documented. The literature presents different types of regression models that are

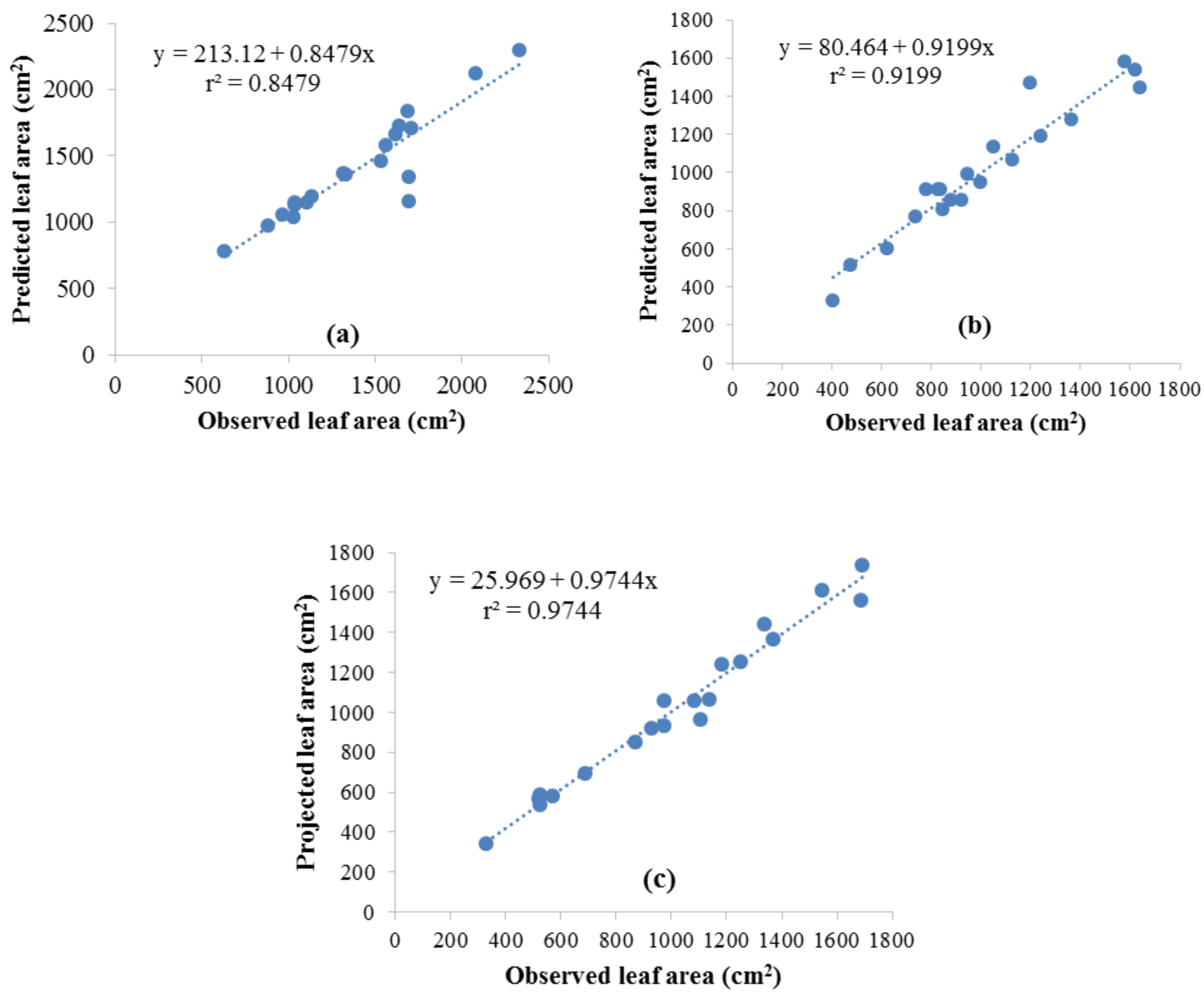

Figure 1. Relationship between predicted and observed leaf areas of *Cordia millenii* Bak. (a), *Gmelina arborea* Roxb. (b), and *Entandrophragma anglolense* C.DC. (c).

used in the process, including power (e.g. Misle *et al*., 2013), second-order polynomial (e.g. Mazzini *et al*., 2010) and linear (e.g. Ogoke *et al*., 2015) models. In this study, linear regression models were adopted because they are simpler than non-linear regression models and have yielded reliable results for a great number of plant species. The linear relationships between observed leaf area and linear functions of L or W, their squares and products were highly significant for each species (Table 3). Overall, 60 - 97% of variations in leaf area were explained by the leaf linear measurements and their derivatives. The regression with LW^2 had the highest r^2 for *C. millenii*, suggesting that the leaf area of this tree species can be estimated with the use of the model $LA = 471.59 + 0.10LW^2$ (Table 3). The selection of the appropriate model for *G. arborea* and *E. angolense* was not as direct because two equations coincided in yielding the highest r^2 (Table 3). The independent variables in the latter equations were W and W^2 for *G. arborea* and LW and LW^2 for *E. angolense* (Table 3). However, the models involving W and LW had lower RMSE, highlighting $LA = -972.63 + 110.90W$ and $LA = -31.81 + 2.88LW$ as the models for a reliable and non-destructive estimation of leaf area of the species (Table 3). The three linear regression models explained 85%, 92%, and 97% of variations in leaf area in *C. millenii*, *G. arborea*, and *E. angolense*, respectively. The finding that a single linear measurement can be used for predicting *G. arborea* leaf area is in agreement with the works of Flavio and Marcos (2003) and Ogoke *et al*. (2015). According to Brito-Rocha *et al*. (2016), the use of a single measurement (L or W) could save up to half of the time that is required for the measurement of both L and W.

Regression analysis and F-test showed a highly significant (P = 0.00) relationship between observed and predicted leaf areas for all three tree species. The high positive values of r^2 associated with the latter models (Figure 1) lend credence to the conclusion that the linear models developed in this study can provide reliable estimates of leaf area of *C. millenii*, *G. arborea*, and *E. angolense*.

CONCLUSION

Linear regression models have been developed for predicting leaf area of *Cordia millenii* Bak., *Gmelina arborea* Roxb., and *Entandrophragma angolense* (Welw.) C.DC. The functions to be put into the equations can be obtained by simple measurement with a ruler. It is, hence, possible to determine the leaf area of these important tree species in a cheap and practical way. The findings of this research can have important implications for forest management given the salient role that leaf area measurement plays in assessing physiological fitness and competitive ability of plants in plantations and natural forests. However, only seven independent variables were tested in this study. Since several other combinations of L and W are mathematically possible, further studies with additional formulae are encouraged.

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